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A PHYSIOLOGICAL STUDY OF GROWTH AND REPRODUCTION AMONG CERTAIN GREEN ALGAE.*†

LEWIS HANFORD TIFFANY

Department of Botany, The Ohio State University.

INTRODUCTION.

Within the last decade or two the algæ have assumed a position of increasing importance because of their relation to aquatic animals, particularly the fishes. There can be no question that for the most part fishes are dependent upon the algæ as direct or indirect sources of their food and energy. The writer (34) has previously called attention to the present and future possibilities of the algæ as indirect sources of food and energy for the human race through the game fishes. The time may speedily come when fishes shall be more generally used as human food, than at present.

It appears, therefore, that any study which increases our knowledge of the fundamental processes and structures of the algæ not only gives us a better conception of the plant kingdom in general but also is a stepping stone toward a scientific cultivation of these plants for fishes.

In reviewing literature on the physiology of the algæ, one notes that two kinds of investigations have been carried on to ascertain (a) the effect of environmental changes on variations in growth and reproduction, and to a smaller degree (b) the nature and kind of substances composing cell walls, protoplasts, stored foods, and pyrenoids. There remains a rather important phase of the subject to extend and correlate these two kinds of work, and to discover what are the actual changes that take

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place in the alga from germination to spore production. For the last three years the writer has made some attempt to correlate, as far as possible, the interrelations of the vegetative and reproductive phases of some of the green algæ with related physiological processes and the changes in the factors of the environment that affect them.

For the sake of ease and clarity of presentation, the results of this work are here recorded under five divisions. It will be convenient, however, to mention some of the work of previous investigators directly in connection with the results of my own work, somewhat regardless of these five major divisions:

I. Review of literature.
II. The cell walls: nature, formation, and variation during growth and reproduction.
III. Protoplasmic inclusions of the nature of food reserves.
IV. Mineral salts.
V. Some environmental factors and their effect on II, III, and IV.

I.

REVIEW OF LITERATURE.

European and American workers have been interested for a number of years in the forms assumed by the green algæ during vegetative development and the initiation of reproductive activities. Their investigations, for the most part, have consisted in calling attention to the various phenomena of reproduction among the algæ and in ascertaining the correlative environmental changes, causative or coincidental.

Pringsheim (29) as early as 1860 had noted that the cell produced by a zoospore among certain Ulothrichales may produce other zoospores immediately upon the attainment of full size, or earlier; or it may pass through a quiescent stage before germination.

Klebs (18) in 1896 observed that *Stigeoclonium tenue* produced round rather than elongated cells in concentrated solution of Knop's nutritive medium. The same author made extensive observations on various species of Vaucheria, Hydrodictyon, Spirogyra, and Draparnaldia to ascertain the effect upon sexual and non-sexual reproduction of such conditions as nutrition, moisture, light, temperature, chemical composition

of the medium, and oxygen. In some species it was possible to produce either sex organs or zoospores almost at will and to produce predominately one or the other. In other species, however, no changes could be effected artificially.

Livingston (22) in 1900 in growing a species of Stigeoclonium in Knop's solution came to the conclusion that variations in the form of this alga were due to variations in osmotic pressure of the medium, independent of the chemical nature of the salts. He says that a high osmotic pressure in the external medium means (a) decreased vegetative activity, (b) an inhibition of zoospore production, (c) a change from cylindrical to spherical cells, and (d) allows for the plane of cell division to be less restricted. A year later the same author (23) explained the change in state from cylindric to spherical cells as being due to changes in water content of the cells; further, that such polymorphism is not related to the photosynthetic process; *i. e.*, darkness has no effect, one way or the other.

A few years later Artari (1) and Matruchot and Molliard (25) found that *Stichococcus bacillaris* in strong sugar solutions produced long, twisted cells, in filaments; in weak solutions there occurred short thick cells, usually isolated. Thus, diametrically opposite results are secured with Stigeoclonium and with Stichococcus, when grown in media of low and high osmotic pressure.

In 1900 Pierce and Randolph (28) working in Italy on "irritability in algæ" observed that the degree of roughness and the character of the surface with which zoospores come into contact determined the extensiveness of holdfast development subsequent to germination; that even among the so-called "non-attached algæ" holdfasts may form if the proper "contact stimulus" be supplied. The authors conclude (a) that the zoospores of Oedogonium come to rest more as a response to the intensity and direction of light than to any other factors; (b) that germination of the zoospores is induced primarily by an interference with their locomotion; (c) that forms and extensiveness of attachment (holdfasts) are determined by the roughness of the surface with which the zoospores come into contact; and (d) that the direction of the growth of rhizoids is always negatively phototropic. Fritsch (10) a little later reported that the zoospores of *Oedogonium capillare* sometimes germinated before actually coming to rest.

Rayss (30) made extensive studies of *Cœlastrum proboscideum* to determine the environmental factors related to changes in state of that alga. He observed that Cœlastrum under normal conditions produced cœnobes. With increasing concentration of nutrient media, isolation of the cells of the alga occurred; abundant nutrition meant large cells and large numbers of cells in the cœnobe; lack of oxygenation caused disarticulation of the cells in the cœnobe; abundant oil formation resulted if the medium were peptone; calcium salts in the proportion of .25 to 1.75 per cent greatly favored the production of cœnobia, while salts of potassium of a similar concentration inhibited cœnobic development; an alkaline medium seemed most favorable to the development of this species of Cœlastrum.

From another point of view several investigators have recorded results of chemical investigations upon the nature of the cell wall and of the protoplast of the green algæ.

West (41, 42) says the cell walls of most green algæ are made of cellulose, sometimes largely of pectose; in all cases that the wall is a secretion of the protoplasm arising on the outer surface of the protoplast. Sometimes cellulose and pectose are sharply differentiated, either in distinct layers or not. The cellulose compounds are usually very compact, surrounded by the gelatinized pectic compounds. Careful investigation by West and Hood (43) shows that Trentopohlia, an alga related to Ulothrix, has an apical cap composed entirely of pectose. The cell walls are lamellose, and the lamellæ are cellulose. Virieux (40) in 1910 found evidence of callose in the mucilage of certain Chlorophyceæ. Lemaire (20) records schizophycose in the sheaths of the Stigonemaceæ. Crow (8) thinks that cell membranes of algæ differ in some ways from those of the higher plants.

According to Lemmermann (21), cellulose occurs in greater or less amounts in all cell walls and goes into solution in concentrated sulphuric acid and in copperoxide-ammonia. This author gives numerous color tests for pectin, starch, sugars, proteins, fats, and oils found in the algæ.

Oltmanns (27) records that of all cell walls those of the algæ are the most variable. He does not believe that the "cuticle" of the Zygnemaceae is identical with that of higher plants, but assigns no reason for his position. Upon plasmolysis, the protoplasts of Zygnema secrete a new membrane around the contracted protoplasm, supposedly of cellulose.

Hirn (15) described the formation of the so-called apical caps in Oedogonium by the development of a circular ring of cellulose just within the upper extremity of the cell, which upon enlargement, ruptures the old wall, forming a ring at the apex as cell division occurs. The author made an attempt to control this development experimentally.

Hodgett (16) described a new species of Spirogyra with peculiar rod-shaped structures in the cell walls at right angles to the length of the filament. He did not discuss their formation, structure, or significance. Similar structures have been noted in *Spirogyra submaxima* by Transeau.

In the formation of zygospores and aplanospores in the genus Debarya, Transeau (36) records the presence of pectic materials within the gametangia surrounding the spores. The same author notes a pectose sheath of seventeen microns thickness in *Spirogyra submaxima*. Transeau (37) has further studied reproduction in *Spirogyra illinoiensis* and notes that "conjugation is initiated by the bending of the gametangia and the development of slight prominences on both cells. This is followed by a mucilaginous secretion at the point of contact, which may persist as a ring about the tube for several days after the union of the cells is complete. After contact, the chromatophores become gorged with starch and fatty bodies, the enlargement of the cell continues, but stops in the case of the male cell when the gamete passes over."

Some very interesting studies of the Myxophyceæ have been made by such workers as Lemaire (20), Griffiths (12), Crow (8), and Mameli (24). The blue-green algæ resemble the greens in some ways, but in others are markedly different. The writer has in progress some investigations on this group of algæ, which will appear in a subsequent paper.

II.

THE CELL WALLS.

1. Methods of Attack.

It has been repeatedly stated by various authors that the constituent materials of cell wall membranes among the algæ were in some manner different from those of the cells in the higher plants. Such expressions as "fungus-cellulose," "chitin-cellulose," and "schizophycose" are indicative of a feeling on

the part of the investigator that such substances have peculiar chemical or physical properties unlike the ordinary substances found in plants. Cell walls vary considerably in the degree and ease of solubility of their constituent parts as well as in permeability to chemical reagents. Wurdack (44), working in this laboratory, found that after removing the outer chitinous layer of *Cladophora glomerata*, the ordinary tests for pectose and cellulose could readily be made. Many of the larger Zygnemaceæ can be immersed in copperoxideammonia for weeks with apparently no change in the cellulose membranes. After treatment for the removal of the pectose layer, however, the cellulose is dissolved in a very few minutes, sometimes almost instantaneously, in the same reagent. The middle wall of the spores of certain species of Spirogyra has deposits of chitin, and this greatly lengthens the time required for the cellulose of this layer to go into solution. After the removal of the chitin, the cellulose dissolves readily. It would seem, then, that the difficulty of ascertaining the nature of the cell walls in the green algæ lies not in the presence of peculiar compounds of strange chemical composition, but rather in the presence of layers of different membrane substances.

Color reactions are not always satisfactory, on the whole, with the majority of algæ unless cross sectioned material is available. The cells of most filamentous algæ are cylinders with the layers in the cell walls one outside the other, and color restrictions to definite areas cannot be detected with any degree of certainty. Solubility tests are most reliable either directly or upon crystals of precipitation. Among the higher plants, if both pectic compounds and cellulose are present, either may be dissolved leaving the other intact. In the species of Zygnemaceæ studied, it is absolutely necessary that the outer layer of pectose be first removed; otherwise, as noted above, the impermeability of the pectose prevents the cellulose-dissolving reagent from coming into contact with the cellulose, or else allows only slight diffusion through itself.

Algæ preserved in 6–3–1* are readily available for most microchemical analyses. A comparison of the same species of Mougeotia, Zygnema, and Spirogyra after being kept in this preservative for a number of years with the freshly collected

* Six parts water, three parts 95% alcohol, and one part formalin.

material showed practically the same reactions toward chemical tests. It must be borne in mind, however, that as a rule preserved material reacts more slowly both as regards solubility and color reactions; otherwise no differences could be discerned. If pectic acid form the larger part of the gelatinous matrix, as for example in some stages of Tetraspora and Palmodictyon, the material undergoes considerable dissolving in the preservative. To be able to correlate the nature of compounds, as well as the amounts, with the growth of the alga, it is desirable to have freshly collected material.

In my work upon the cell walls of certain green algæ the standard microchemical tests have been used. With restrictions and modifications noted above, the order recommended by Tunmann (39) is good:

a. Color reactions.
b. Solubility.
c. Hydrolysis and tests for hydrolytic products.
d. Precipitation.
e. Crystal formation.
f. Optical properties.

Since the membrane materials are often delicate and deposited in small quantities, it is quite essential that the investigator "be on hand" when the reactions are taking place. The cell walls of *Microspora Willeana* under polarized light are almost wholly bright, due to the prominence of the cellulose. If copperoxideammonia be applied, the cellulose may swell to such an extent as to burst the pectose layer on the outside, later dissolving. One might conclude that the cell membrane contained nothing but cellulose. The middle layer of the spores of many of the Zygnemaceæ will after a time disappear in copperoxideammonia, but if one notes the difficulty of solubility and applies other tests, he finds deposits of chitin.

It should also be emphasized that tests must be repeated many times if one's conclusions are to be really conclusive. A filament of *Tribonema bombycina* when placed in dilute potassium hydroxide was observed to "peel off" layer by later and go into solution—pectic acid. Other filaments in warm 2% hydrochloric acid followed by 2% potassium hydroxide disappeared entirely, but were unaffected by the potassium hydroxide alone—pectose. Still other filaments were observed to

disintegrate slowly and imperfectly into H-pieces and finally dissolve in copperoxideammonia—cellulose. If one were to take these tests separately and alone he would find the membrane in one case to be pectic acid, in another pectose, and in still another cellulose. This variation is somewhat a matter of age, the older filaments having relatively less pectic compounds and a greater amount of cellulose.

By the use of a small piece of blotting paper adjacent to one side of the cover glass, it is possible to get reactions of a single alga to various reagents which can be "drawn through" from the other side of the cover glass, one after the other. This not only saves time, but makes it possible for one to observe all the changes in the individual alga and to be assured it is the same alga under continuous observation.

In the selection of material for experimental work it was necessary to pay considerable attention to those species of algæ which were accessible at various seasons of the year. To get fresh specimens in the winter time, recourse was had to those forms growing in water tanks in the botany greenhouse. Most of the algæ used are common in Ohio. This report does not attempt to give complete analyses of any group or groups of algæ, neither does it strive to point out relationships among these groups, as such. The aim has been to select individuals representing essentially diverse taxonomic categories among the Chlorophyceæ, thus laying the foundation for more specific work among certain groups or genera at a later time.

2. The Zygnemaceæ.

The Zygnemaceæ are represented in Ohio by four distinct genera—Mougeotia, Debarya, Zygnema, and Spirogyra—differing from each other chiefly in the nature of the protoplast and in the method of reproduction. The family is world-wide and constitutes some of our commonest freshwater algæ. The thallus is filamentous, consisting of a single series of cells that form an unbranched filament. The nature of the cell walls and notes on growth and reproduction of these algæ follow under each genus.

a. Mougeotia.

In the genus Mougeotia the cell wall in the vegetative state is entirely of cellulose, surrounded by a thin mucilaginous

investment which is, in all cases studied, of pectose. In weak hydrochloric acid warmed over a water bath for twenty to thirty minutes, followed by warm alkalies, the pectose is easily soluble. When the pectose layer is removed, the cellulose membrane dissolves away very readily upon the application of copperoxideammonia or 60% sulphuric acid. On the other hand, if freshly collected material be subjected to treatment with copperoxideammonia, several hours are usually required for the penetration of the pectose layer by the reagent. Material preserved in 6–3–1 must first be treated for the removal of the pectose layer before copperoxideammonia will penetrate through the pectose to the cellulose. Filaments of *Mougeotia robusta* in the copperoxideammonia for five weeks were unaffected.

The middle lamella formed from the first transverse division laid down in the cell is pectose. Just previous to fragmentation of the filament the lenticular area of pectose becomes pectin, which upon dissolving leads to the separation of the adjacent cells at the plane of the original lamella. This fragmentation may occur between all the cells of the filament, or irregularly throughout the thallus.

The formation of zygospores in Mougeotia from only a part of the protoplast of the gametangium institutes wall formation peculiar to the genus. Partition walls of cellulose cut off the zygospore from the rest of the gametangium, and later other membrane layers are deposited within the first partition. These differentiated layers are the wall of the zygospore. The outer layer, which is the first one formed, and the inner layer are of cellulose. The middle layer (sometimes layers) is usually quite distinct from the other two. It is usually isotropic in polarized light, insoluble in weak acids, and very difficultly soluble in copperoxideammonia. Concentrated chlorzinciodide causes the appearance of a faint violet color in the membrane, finally disappearing. The red-violet color indicating chitosan is usually clear, sometimes very definite, and occasionally lacking. These phenomena indicate that the middle layer is not pure cellulose but contains deposits of chitin. There is no evidence of a definite chitinous layer, but the amount of chitin present may vary from none at all to a considerable quantity. The amount of chitinous deposits varies not only with the different species examined, but in the spores of the same filament. It is interesting to note that the markings of the spore wall, whose presence

or absence is often an ultimate criterion of species identification, are present only on this middle layer. The warts of the spores of *Mougeotia quadrangulata* are cellulose with varying amounts of chitin.

Mougeotia genuflexa exhibits a peculiar "contact-formation" between cells of the filaments that causes the amateur much fruitless search for the zygospores supposedly appearing subsequent to this apparent conjugation. Accompanying the genuflexing is the separation of the "knee-joints" from the rest of the filament by the formation of soluble pectin at the region of the middle lamella. Actual communication apparently does not result from such contact, although stages are common in which the appressed walls are completely fused into one. Nieuwland (26) thinks that this occurrence is merely a step in the process of vegetative multiplication. Kneeing is probably the result of changes in the chemical nature of the cell wall accompanied by internal pressure. There is a rapid increase in the amount of pectose at the region of the "bulge," and when these pectose areas from different filaments come into contact, the two knees are "glued" together. Oftentimes the cellulose layers never come into actual contact, being separated by the pectose layer; sometimes the cellulose layers come into contact, but never fuse; or there may be complete fusion, the appressed region acting as one cell wall. In some cases, particularly when the alga is growing in a 5% solution of sucrose, there occurs a breaking up of the protoplasm and a movement of the disintegrated protoplasts towards the points of contact. Nothing nearer than this to actual fusion of gametes was ever observed, and further movement could not be induced artificially.

The above report regarding the genus Mougeotia is based on a study of the following species: *Mougeotia quadrangulata*, *M. robusta*, *M. robusta biornata*, *M. calcarea*, and *M. genuflexa*.

b. Debarya.

In the genus Debarya the zygote is the result of the fusion of gametes formed from the entire contents of the gametangia. Chemically the walls of the vegetative cells are identical with those of Mougeotia and Zygnema. West (41) in describing the formation of the zygospore speaks of the laying down of a series of cellulose layers resulting in a much thickened gametangial wall. In *Debarya decussata* this is accompanied by

deposits of chitin. The highly refractive area formed in the gametangia upon the maturing of the zygospores has been supposed to be of pectic compounds. Careful investigation upon the filaments of *D. decussata* failed to show any pectic compounds in this area, but the cellulose tests were positive. Further work is desirable on other species of this interesting genus.

c. Zygnema.

The genus Zygnema is very similar chemically to Mougeotia and Debarya. The external pectose sheath in *Z. pectinatum* may reach a thickness of nine microns. The cells of the filaments of Zygnema are usually shorter and thicker than those of the two genera mentioned above. The spores are formed from the entire contents of the gametangia and consist of three wall layers, all of cellulose with the middle one often chitinized.

As reproduction is initiated in Zygnema it not infrequently happens that there is incomplete fusion of the gametes in zygospore formation, and two "spores" result. In some species of the genus, aplanosporic formation is common. In either of these cases the spore walls are formed in the same way as in the ordinary zygote. Upon plasmolysis, either natural or induced artificially, the protoplast secretes a new wall of cellulose within the old cell wall.

In *Z. insignis* the mature zygospores have deposits of chitin in the middle layer and the outer spore membrane is often entirely of pectose. Other species with chitinous deposits in the middle spore membrane are *Z. stellinum*, *Z. pectinatum*, *Z. collinsianum*, and *Z. decussatum*.

Hodgett (17) has made careful observations of the conjugation of *Zygnema* (*Zygonium*) *ericetorum*, which in some ways resembles that of *Spirogyra majuscula*, noted below. He says that cells of adjacent filaments put out protuberances that meet and become flattened against each other, mucilage being developed from the outermost layers of the cell walls. The protuberances are due to the more active growth of the innermost layer, the outgrowth being thickened by deposition of new internal layers of cellulose. The alga exhibits a rather unique peculiarity prior to the fusion of the gametes in that a thin wall of cellulose forms around each gamete. Later, the line of separation between the two gametes is lost, fusion occurs,

and an ellipsoidal zygospore is formed. The thin inner wall of each gametangium remains around the corresponding half of the zygote, the two membranes becoming fused together and forming a continuous wall. The investment so formed persists as the outer wall of the zygospore. Layers are subsequently deposited internally, resulting in a lamellated wall at maturity.

d. Spirogyra.

An examination of some twenty species of Spirogyra shows a remarkable similarity in the chemical composition of the vegetative cell walls and those of the zygospores, with the exception of the Sirogonium division of the genus, which will be described separately. In all species studied the filaments proper are surrounded with a mucilaginous sheath of pectose, varying considerably in thickness with the different species. In *S. flavescens*, *S. Weberi*, and *S. catenaeformis* it is very thin and easily overlooked. With the species of larger diameter the amount of mucilaginous material is usually correspondingly increased. The thickest pectose sheath is found in *S. submaxima*, sometimes reaching a thickness of seventeen microns. Its permeability to reagents varies somewhat with age and water supply, but the exact factors of the environment responsible for such variation are still under investigation.

The cell wall beneath the pectose envelope is apparently in all cases composed of two layers, the innermost thin and delicate, covering the protoplasts; the outer is thicker and tougher; both are of cellulose. The replicate end walls of such species as *S. Weberi*, *S. protecta*, and *S. insignis* are merely annular ingrowths of cellulose which lead to fragmentation of the filament by becoming everted, as noted by West (41).

Stages antecedent to and accompanying conjugation have been noted in *S. majuscula*, collected in a roadside rivulet in July, 1922, and kept in aquaria. Outgrowths of cellulose occur usually about halfway between the ends of the conjugating cells. When these protuberances from adjacent filaments come into contact, the ends fuse, and disintegration of the planes of contact occurs soon after, resulting in a conjugation tube. Just what chemical changes occur in the absorption of the fused ends has not been followed in detail. It appears that the dissolved cellulose accompanies the male gamete into the female gametangium, entering into the formation of the

zygote. The conjugation tube is soon invested with a pectose covering and behaves as the rest of the filament toward color reactions and solubility tests.

In the formation of the zygospore after the fusion of the gametes, there is a further contraction of the fused protoplasts until they occupy a position about the center of the cells. At first only a thin transparent wall, surrounding the fused protoplasts, can be noted. Within one to three days the wall is seen to be composed of two layers, the outermost one considerably thicker than the inner. Upon the application of copperoxide-ammonia, the outer layer swells and occupies a volume from one and a half to six times its original size. As it goes into solution, (after fifteen minutes to one hour) the inner layer dissolves too, leaving a naked protoplast of contracted spirals. Within a week or ten days the spore is mature, and besides having another layer, it has taken on other properties. The outer layer and the inner layer are entirely of cellulose. The middle layer, very difficultly soluble in copperoxideammonia and isotropic in polarized light, often has deposits of chitin. In the rough-spored species examined, the spore markings were always associated with chitinous deposits. It is difficult to explain the irregularity deposited areas of chitin, for even in the smooth-spored species there is no continuous layer of chitin.

In the germination of the zygospore the outer walls are burst through by the developing sporeling. The inner membrane of the spore becomes the wall of the developing sporeling. In making microchemical analyses of the germinating spores one gets a definite test for chitin, but one must not incorrectly interpret this to mean that chitin is present in the young vegetative plant. Tests indicative of chitin were in every case the result of the reagent coming into contact with the middle layer of the old zygospore. Within a few days a pectose layer is noted around the sporeling, and repeated cell divisions result in the formation of a filament.

Separate mention should be made of *Spirogyra illinoiensis* and *S. stictica*, which belong to the Sirogonium (Choapsis) division of the genus. There is no real conjugation tube, but the walls of the gametangia come into contact, giving the filaments a genuflexed appearance similar to that described for *Mougeotia genuflexa*. A perforation is effected at the plane of contact by dissolving the cellulose and the contents of one

gametangium, after having completely disintegrated, pass over into the other. The fusion of the two results in a zygote.

Conjugation is not effected in all of the cells of the filament. Among certain cells there is noted a considerable decrease in size in direction of the length of the filament. The cell walls undergo slight thickening, and an increased amount of pectose is noted in the vicinity of the plane of contact of the two gametangia in apposition. Upon the complete coalescing of the two gametes in the female gametangium, a heavy somewhat differentiated wall is formed at the periphery of the fused protoplasts inside of, and not connected with, the wall of the gametangium. This wall is composed of three or more layers or coats. The outer one, anisotropic in polarized light, is usually pectose and can be completely removed after heating for one hour in 3% hydrochloric acid over a water bath and following this with warm 2% potassium hydroxide. The remaining layers of the wall are largely cellulose, but with considerable variation in solubility and reaction to polarized light. Upon the application of copperoxideammonia, an outer anisotropic layer dissolves in a few minutes. The remaining layer (sometimes layers) swells, is ruptured, and comes off the zygote like the covering of a much-used baseball. This shell is anisotropic in polarized light, is insoluble in cold concentrated sulphuric or hydrochloric acid, and fails to give the hydrocellulose reaction. If, however, the membrane remains in copperoxideammonia for from fifteen to twenty-four hours, it is completely dissolved. A further analysis of this membrane as it is dissolving shows that it has deposits of chitin, similar to that of the spores of the species of Spirogyra described above.

It is interesting to note that the gametangial walls behave almost exactly as the membranes of the zygospore. Upon the dissolving of the pectose membrane, the polarizer reveals a clear light layer surrounding a darker one. The outer is pure cellulose, but the inner has irregular deposits of chitin. The walls of the vegetative cells not concerned in zygospore formation are chemically identical with those of the other species of Spirogyra not belonging to the Sirogonium division.

In these two members of the genus two notable chemical differences occur. The zygospores have an outer layer of pectose and the fruiting cell becomes slightly shortened and the wall thickened and changed to the extent that the inner mem-

brane becomes chitinized. This together with their peculiar method of conjugation makes them an interesting division of the genus. West (41) has recommended that the generic name Sirogonium be retained for these forms. In view of the fact, however, that in many species like *S. tenuissima*, *S. punctiformis*, *S. circumlineata*, and others, the female furnishes little or no protuberance; that the gametangial walls of *S. ellipsospora*, *S. hydrodictya*, and *S. crassa* undergo changes in size and shape, as well as cell thickness, and that chitinous deposits in the gametangial walls are slight and sometimes wanting—it does not seem necessary or desirable to increase further the taxonomic nomenclature by providing another genus among the Zygnemaceæ for these variations.

Species of Spirogyra examined were the following: *Spirogyra varians*, *S. circumlineata*, *S. catenaeformis*, *S. flavescens*, *S. velata*, *S. daedalea*, *S. irregularis*, *S. ellipsospora*, *S. ellipsospora crassoidea*, *S. fluviatilis*, *S. novae-angliae*, *S. crassa*, *S. majuscula*, *S. hydrodictya*, *S. Weberi*, *S. laxa*, *S. tenuissima*, *S. tenuissima fovealata*, *S. inflata*, *S. protecta*, *S. rectangularis*, *S. insignis*, *S. stictica*, *and S. illinoiensis.*

3. Microsporaceæ: Microspora.

The filaments of Microsopra are cylindrical and unbranched, and the character of the wall varies with different species. In *Microspora Willeana* the wall is distinctly lamellate, being constructed in such a way that upon disarticulation of the filament, H-pieces are formed. Each H-piece consists of two cup-like cylinders having a common base which forms the transverse wall of the filament. Each cylinder concavely thickened at its base becomes thinner toward the top. The fitting together of the H.-pieces by the dove-tailing of these thinner parts forms the filament, so constructed that from an internal view a slightly concave H-piece alternates with one slightly convex, giving the whole filament a nearly cylindrical shape (Fig. 1). Thus each uninucleate cell is composed of two halves of supplemental H-pieces.

A microchemical analysis of *M. Willeana* shows that the reticulated chloroplast is surrounded by a thin wall of cellulose which, though appressed to the conjoined halves of two separate H-pieces, behaves as a unit. It can be noted after disarticulation of the filament, but it dissolves very quickly in copper-

oxideammonia. It gives a bright line with polarized light. The H-pieces themselves consist of more difficultly soluble cellulose, the overlapping pieces of which are connected by a very thin membrane that varies considerably in structure and nature. The whole is surrounded by a thin mucous coat of pectic acid and pectose.

The pectose layer on the outside sometimes extends inward for some distance between the appressed H-pieces, so that upon treatment with warm hydrochloric acid and dilute potassium hydroxide this inward protrusion is dissolved. As a result the filaments appear as in Fig. 2. There is a corresponding outward protrusion of the inner cellulose layer between the H-pieces (Fig. 2a). Continued immersion in copperoxide-ammonia for three to six hours causes the swelling of the inner cellulose wall with its outward protrusion, and the filament is ruptured, breaking up into H-pieces. And as if this were not sufficient complication, there is sometimes a small amount of calcium pectate connecting the outward extension of the cellulose and the inward protrusion of the outer pectose layer. There exists considerable variation in the amounts of these compounds. Often it is almost wholly cellulose, sometimes mostly pectose, and rarely of calcium pectate.

It is interesting to note that copperoxideammonia penetrates readily through the pectose and pectic acid layer, first swelling and finally dissolving the cellulose membrane. If the pectose and pectic acid be first removed, the whole filament appears bright under polarized light. Upon the application of cellulose-dissolving reagents, the remainder of the thin layer separating the dove-tailed H-pieces is dissolved, resulting in the disarticulation of the filament. The cellulose surrounding the protoplast then dissolves rapidly away—in fact almost as soon as the reagent strikes it. The H-pieces swell considerably, particularly at the transverse wall, and in from two to ten minutes are completely dissolved.

The formation of these peculiar H-pieces can be observed by watching the development of the plant during cell division. As soon as the cross wall is laid down, additional concave layers of cellulose are deposited internally at the juncture of the partition with the peripheral cell wall. A sporeling, having made its first cell division, as above, would consist of one H-piece the extremities of which are connected as in Fig. 3. The next

wall (or walls) formed similarly presents a formation like that shown in Fig. 4. Now upon the formation of a cross wall at *a* or *b* (Fig. 4), the concave thickenings are laid down, and as their extremities must necessarily underlap the extremities of the thickenings previously laid down, the latter H-piece is in reality within the cylinder formed by the halves of the older adjacent H-pieces. Upon the growth of the new H-piece the outer cylindrical shell is distended to breaking, the rupture usually occurring obliquely to the length of the filament. This new H-piece develops slightly concave inwards. Thus are formed a series of H-pieces, consisting of a piece slightly concave alternating with one slightly convex, seen from the inside of the filament. Further cell division in this region of the filament was not observed. Continual cell division at either extremity of the sporeling, as described above, accompanied by the deposition of concave thickenings of cellulose on either side of the new partition and consequent growth in length of the peripheral wall, forms a many-celled filament.

4. Cylindrocapsaceæ: Cylindrocapsa.

Another interesting genus of the Ulothrichales is Cylindrocapsa, of which *C. geminella* and the variety *minor* are quite common in Ohio. The filaments consist of ellipsoid cells frequently grouped in pairs and provided with a cell wall greatly lamellated. The whole filament is inclosed within a rather tough sheath of pectose and pectic acid, the latter being more abundant in the younger thalli. The lamellæ, however, are of cellulose and not pectic in nature. The cell wall proper is of cellulose more difficultly soluble than the apposed lamellæ, which strip off easily one after the other in cellulose-dissolving reagents.

5. Tribonemaceæ: Tribonema.

The filaments of Tribonema are apparently constructed much in the same way as are those of *Microspora Willeana*, previously described. Dovetailing is not so marked in Tribonema, and the cell walls are relatively thinner; but the characteristic H-pieces appear upon the disarticultation of the filament. A long H-piece usually alternates with a shorter one, the extremities of the latter being situated internally to those of the former. Bohlin (3) figures the cell wall as composed of

several apposed layers of pectic compounds. In polarized light the filament is usually dark and only rarely touched by the application of copperoxideammonia. The outer layer is uniformly of pectose and the others may be either pectic acid or pectose, very rarely cellulose. There is no indication of calcium pectate in the filament. It appears that the conjoined H-pieces are held together sometimes by pectic acid, but more often by pectose of a slightly greater solubility than the rest of the layers in warm dilute acids and alkalies.

Species examined were: *Tribonema bombycina*, *T. bombycina tenue*, *T. utriculosa*, and *T. minus*.

6. Cell wall formation in relation to algal epiphytes.

Algal species, epiphytic upon other algæ, are rather common among members of the Chlorophyceæ, as well as among the Myxophyceæ and Bacillariæ. The manner of epiphytism, while variable in form and degree, usually assumes one of four relations to the host plant: (a) gelatinous stalks, either simple or branched, of pectose secreted by the so-called stipitate diatoms; (b) holdfast cells (haptera) which arise as basal cells in the germinating spores of such algæ as Oedogonium, Bulbochæte, Microspora, Chætophora, and more rarely in some species of Spirogyra and Zygnema; (c) each cell of the alga either unicellular, filamentous, or thalloid in direct contact with the host, as in Cocconeis, Chamæsiphon, Chætosphæridium, and Sykidion; and (d) mere attachment to a plant or other substratum of such more or less macroscopic mucilaginous colonies as Tetraspora or Apiocystis. The mucilage here may be either pectic acid or pectose; and the two are sometimes present somewhat intermixed.

The pectose stalks of some of the diatoms are found usually only in the early spring. Sudden rises in temperature, which in some way hasten the conversion of pectose into water-soluble pectin, account for the pulses of diatoms in rivulets and streams, as the writer (34) has noted in another paper.

Oedogonium and Bulbochæte normally have cell walls in three layers, viz.: the outermost chitin, the middle one pectose, and the inner cellulose. The basal cell, however, is not uniform in this respect. That part of the holdfast wall directly in contact with the algal host has two layers, instead of three. Here the mucilaginous pectose, forming the middle layer of the cell

wall elsewhere on the filament, forms the outer layer and is directly appressed to the wall of the host. The inner layer is unchanged (Cf. Fig. 5).

In the dioecious nannandrous species of the Oedogoniales the male plants are epiphytic on the female plants. The androspores come to rest on the wall of the oogonium, the suffultory cell, or sometimes other cells and produce upon germination dwarf males, or nannandria. The basal cell of the dwarf males (or the whole nannandrium if it be unicellular) is attached to the female plant in precisely the same way as the holdfasts are attached to the host plant, as noted above (Cf. Fig. 6).

Hirn (15) in the drawings of his magnificent monograph of the Oedogoniales invariably shows that the outer layer of the wall of the basal cell does not extend all the way around the holdfast. He does not note, however, that the discontinuance of the outer cell wall layer is significant. This detail in his sketches is simply another evidence of the wonderful keenness of his observations.

The epiphytes can be detached from the host plant in all cases if the algæ are placed in pectose-dissolving reagents. The brownish-red salt of iron, as reported by Fritsch (11), deposited about the attachment surface of the basal cells of some algæ may or may not be present, and as far as my observations go contributes nothing directly to the attachment of the epiphytes.

Algal epiphytes are often found attached to species of Oedogonium, Bulbochæte, Cladophora, Pithophora, and Vaucheria at any time during their life cycle. These same epiphytes, if they occur at all, are not found on Spirogyra, Zygnema, or Mougeotia during their period of vegetative growth; that is, attachment to these algæ occurs normally only after the initiation of reproductive activities. The explanation is rather evident when one compares the cell wall composition of the two groups of host plants mentioned above. In the Oedogoniales the outer layer of the filament is usually chitin, while in the last three forms the outermost layer of the cell walls is pectose. The cell walls of the former group do not materially change with age, except in thickness by internal deposition of cellulose. In the species of Zygnemaceæ examined, on the other hand, there appears to be almost a continuous transformation of cell wall material during vegetative development.

There is considerable doubt among algologists as to the manner of formation of mucilaginous pectic compounds in the filamentous Conjugatæ. It has been pretty definitely shown by Klebs, Hauptfleisch, and Lutkemuller (according to West) that in the Desmids mucus is secreted through cylindrical pores which pass outward directly through the cell wall. West (41) inclines to the opinion that in many of the Chlorophyceæ, however, "much of the mucus arises by the conversion of the outer layers of cellulose into mucilaginous substances of varying degrees of solubility in water, and increments are constantly added by the gelatinization of successive layers."

Pores similar to those found in the Desmids are not visible in the cell walls of either Spirogyra, Mougeotia, or Zygnema. No pectose can be detected on the sporeling until after transverse wall formation has begun. As spore formation occurs in these algæ, the amount of pectose present becomes gradually decreased, until finally the cellulose layer is practically exposed. These observations, though somewhat indirect evidence, lend credence to the statement just quoted from West. At any rate, epiphytic algæ have not been observed upon the Zygnemacæ when the pectose layer is present.

The absence of epiphytic algæ from Spirogyra, Mougeotia, and Zygnema during their vegetative growth is explained by the fact that there is a gradual change of pectose into water-soluble pectin during this period. When the amount of pectose formed internally is equal to that dissolved externally, a sort of equilibrium is established, and the thickness of the pectose remains practically constant. When the activity is shifted in either direction, the pectose sheath is accordingly thin or thick. As spore formation begins, the formation of pectose stops. Pectin formation occurs until the pectose is exhausted, exposing the cellulose layer of the cell wall. Obviously, as long as the external pectose-to-pectin conversion takes place, epiphytic stability is decidedly uncertain. The cellulose, thus exposed, undergoes no further change until decay sets in, and epiphytes are found rather commonly at these stages.

III.

Protoplasmic Inclusions of the Nature of Food Reserves.

The principal food reserve in most of the green algæ during the period of their vegetative growth is starch. Exceptions to this general statement are the various species of Vaucheria, under normal conditions, and the Heterokontæ. In these latter algæ the food reserve is a fatty oil. Davis (9) in his historical resume of algological literature bearing directly or indirectly upon enzyme action notes the following: Beyerinck (2) found glycogen in *Chlorella variegata*. Kuster (19) reports crystal formation, probably inulin, in Derbesia and Bryopsis. Swartz (33) concludes that in Enteromorpha the carbohydrates exist as pentosans and galactans. Tihomirow (35), using the phenylhydrazine method, secured after a month yellow amorphous deposits in the cells of *Codium bursa* and *C. tomentosum* (both Siphonales). He could not determine the sugars these osazones represented, but suggested dextrose and *d*-galactose.

The algæ with which this phase of the work has been carried on include the species of Zygnemaceæ named in Part II above, and in addition *Cladophora glomerata*, *Pithophora varia*, *Vaucheria geminata*, *V. geminata racemosa*, and *V. hamata*.

Starches and Oils.—In the Zygnemaceæ and Siphonocladiales studied the starch is principally associated as an integral part of the pyrenoids. Under conditions of rather high photosynthetic activity it may be found outside the pyrenoids in the chloroplasts.

The amount of starch present has a daily as well as a seasonal periodicity. The former depends principally on the availability of sunshine. The latter is associated in some way with the changes in the metabolic gradient accompanying the different phases of the life cycle of the alga. The iodine test of an early morning before sunrise gives a definite reaction for starch only in the periphery of the pyrenoid. After an hour or more of sunshine, the whole chloroplast appears purplish. An examination of the spores of the algæ reveals the fact that in addition to the starch a considerable quantity of oil is present. The comparative percentages vary with the different spores, but the amount of starch present is slightly in excess of that of

the oil. Upon germination of the spore, the oil is used up, and no fats are discernible in the sporeling. During the vegetative growth of the plant, starch is predominantly the food reserve. As soon, however, as the reproductive period of the alga begins to be reached, the amount of oil present in the chloroplasts increases rapidly. The fully formed spore contains fats and starches, from 20% to 50% of which is estimated to be oils.

Carter (6) notes that in the autumn there is an accumulation of starch in the Cladophoraceæ in the form of small grains. The starch is lodged in the interstices of the protoplasmic reticulum of the chloroplasts. Many of the Cladophoras are perennial and carry on photosynthesis whenever there is an availability of sunlight and proper temperature.

The starch envelope has a rather intimate connection with the rest of the pyrenoid. In fact, the writer has not been able to get starch-free algæ in a living condition among the Zygnemaceæ or the Siphonocladiales. Algæ placed in the dark lose starch, as indicated by the lessened intensity of the iodine test. But even after decomposition and decay of the walls and protoplasts have begun, there can be still secured definite tests for starch in the cells.

West (41) has noted that the pyrenoids of the Zygnemaceæ are in the nature of aleurone grains. Treatment of the pyrenoids with a solution of nigrosin-picric acid brings out the protein crystals as yellowish-green and shows the surrounding globoids to be colorless. Around this, of course, appears the starchy envelope.

In species of Vaucheria, under normal conditions, the food reserve is a fatty oil, not starch. Pyrenoids do not exist in the genus Vaucheria, and it has been supposed by some that the oils may be the first synthetic products. Dichotomosiphon, a genus closely related to Vaucheria, stores starch instead of oil. Evidence will be submitted later (See Part IV) to show that starch may under some conditions be the partial, if not the whole, food reserve, even in Vaucheria. The writer inclines strongly to the belief that the various food syntheses among the algæ are not different fundamentally from those of the higher plants. Although some evidence is at hand to substantiate this opinion, the proof does not yet seem sufficiently adequate to present at this time.

Sugars.—It has been a question of interest for some time as to what the first visible product of photosynthesis among the Chlorophyceæ really is. Microchemical and macrochemical tests for sugars in these algæ have been for the most part either negative or so indefinite that the actual sugars concerned are very uncertain. Some attempt has been made to ascertain what sugars, if any, are the first photosynthetic products. Tests were made in all cases upon material brought into the laboratory directly from ponds and streams nearby. This material, previously exposed to direct sunshine, had all evidence of carrying on photosynthesis at a rather high rate.

Upon the application of copper tartrate and 20% sodium hydroxide (Fluckiger's test) to algæ thus collected and washed in distilled water, crystals of cuprous oxide appeared in a short time after heating. The crystals were in the solution, however, and not within the cells. If this test indicates glucose, it appears that the sugar is in a very soluble state and diffuses through the algal walls very readily. Checking up this reaction with other sugar tests gave very indefinite results. Both Benedict's and Fehling's solutions gave copper crystals very tardily, indicating the presence of some reducing agent, but not necessarily glucose. The osazone tests were practically negative. Results similar to those reported by Tihomirow (35) noted above were obtained after periods of from three to five weeks. The sugars these osazones represent are not identifiable.

Sayre (32) working in this laboratory secures somewhat similar results on sugar tests in the guard cells of *Rumex patentia* leaves. He gets cuprous oxide crystals with Fluckiger's, Benedict's, and Fehling's solutions, but has been unable to get osazone formation.

If glucose be the first photosynthetic product in the algæ studied, it must be transformed very readily into starch. It seems that the ordinary sugar tests are not applicable. From the definiteness of the Fluckiger reaction it appears that one of the hexoses is present. The writer hopes to be able to secure some modifications of the ordinary sugar tests that will indicate the smallest quantity of sugars present and help clear up the primary carbohydrate syntheses in the green algæ.

Hemicelluloses.—In the algæ examined methyl pentosans appear to be absent. The mannose hydrazone reaction for mannan was negative. There are distinct color reactions

with the orcin- and phloroglucin-hydrochloric acid tests for galactan and araban, but localization was difficult. These pentosans appear to be a part of the protoplasts.

Tannins.—Tannins are mostly non-crystallizable colloidal substances and are rather generally distributed in plants. Haas and Hill (13) note the occurrence of tannins in Spirogyra, Mougeotia, and Zygnema in the cells in the form of numerous small vesicles. These authors review the work of Van Wisselingh, who concluded from his investigations that (a) tannins played an important role in cell wall formation in certain cases in Spirogyra; (b) cells about to conjugate are rich in tannins; (c) there occurs a gradual diminution of tannin as conjugation proceeds, until the zygospore at maturity contains a mere trace; and (d) upon the interruption of conjugation tannin accumulation continues until the death of the plant.

Tests with a number of species of Zygnemaceæ tend to confirm most of the results noted above, but give no supportive data for others. Tannins are notably absent from these algæ during the period of active vegetative growth. It is during this time that most wall formation occurs, particularly cross walls. In other words, as long as active cell division is occurring, tannin tests are negative. Just as soon, however, as active vegetative growth ceases and reproduction begins, there is an accumulation of tannins. The tannins are most abundant during the development of conjugation tubes and during gamete formation in the Zygnemaceæ. They rapidly diminish during the fusion of the gametes and are practically absent from the mature zygote. It appears that the tannins occur along with changes in the metabolic gradient accompanying the transition from a vegetative state to a reproductive state. That the tannins are used in the elaboration of other materials during spore formation seems certain, but further investigation is necessary to determine the exact use of these substances in the algæ.

Cladophora glomerata, *Pithophora varia*, and the species of Vaucheria examined gave negative results for tannins.

Tests for tannins are easily made with ferric chloride, osmic acid, or a solution of ammonium molybdate in concentrated ammonium chloride.

Inulin.—Tests for inulin gave negative results.

IV.

Some Mineral Elements.

Microchemical tests for minerals are difficult to make in the algæ with any degree of certainty of localization because the entire plant lives practically submerged in water. The presence of a mineral salt within the cell sap of an alga does not necessarily mean that it will enter into the metabolism of the cell. Toxic substances like copper sulphate which are fatal to Spirogyra even in extremely small concentrations are readily taken in through the cell walls. Attention has been directed principally to the mineral salts found in connection with the chloroplasts and the cell walls.

The following brief summary gives the results of investigations to date. More work is in progress, and the subject will be treated more completely at the conclusion of those investigations. Some particular work with certain algæ with artificial environment bears a direct relation to mineral content, and this is discussed under Part V below.

Iron.....Ferric compounds found in the chloroplasts.

Calcium.....In the form of calcium pectate in the middle lamella of the walls of some algæ. Calcium is necessary for colonial and filamentous integration even though a distinct calcium pectate layer could not be noted. The calcium pectate layer can sometimes be determined in the large Oedogoniales and in *Microspora Willeana*. Evidence will be submitted later to show that calcium is necessary in some way for the formation of the middle layer of pectose in some filamentous Conjugatæ.

Potassium.....In many Spirogyras yellowish crystals of potassium chloroplatinate are found upon the application of an aqueous solution of platinum chloride. These crystals are found with difficulty except when conjugation tubes are being formed. Potassium seems to be abundant at the place where new wall formation occurs accompanying conjugation.

Phosphorus.....Species of Zygnemaceæ immersed in a solution of ammonium molybdate in nitric acid shows ammonium-phospho-molybdate crystals in the protoplasts. The crystals are small and the yellowish center is not always evident. In about ten minutes, if washed in dilute hydrochloric acid and treated with a 1% solution of phenyl-hydrazine hydrochloride, a bluish color appears in the chloroplasts.

Magnesium.....Noted sparsely in the cell sap, but not in the chloroplasts.

V.

Some Environmental Changes and their Effects on Algal Growth and Reproduction.

Continuous artificial illumination.—Recently Harvey (14) has attacked the problem of growing seed plants in artificial light. He found that a number of plants grew from seed to maturity and set good seed in continuous artificial illumination. Other plants bloomed but did not set seed. Potatoes produced good tubers.

The writer has had under investigation for some months various species of Cladophora, Pithophora, Spirogyra, Zygnema, Cylindrocapsa, and Oedogonium, exposed continuously to artificial illumination. In this experiment two two-hundred watt Mazda lamps were used over a water area of 4 square feet. The bulbs were two feet from the water with sufficient ventilation to prevent excessive heating. The apparatus consisted of a box two feet square and six feet long placed open end down in a tank of water in the greenhouse. Extending nearly to the top of the water from the bottom of the tank is a concrete post, through which an iron pipe carries water. Water escapes from the tank through the exit pipe. In this way water is kept in circulation, thus aiding in keeping the temperature within the box and without the box nearly constant. This gives excellent opportunity for the study of comparative growths of the same species of algæ in the same water under almost identical conditions, except sunlight is available in the one case, while in the other the algæ are exposed to continuous artificial illumination. The upper end of the box allows for the entrance of the electric connection and at the same time serves as an exit for the heated air.

The occurrence of holdfasts in many of the attached filamentous algæ is common. Transeau (37), Collins (7), West (41, 42), Borge (4), and others report holdfasts as occurring among certain forms of the Zygnemaceæ, which are usually unattached. Pierce and Randolph (28) found that the extensiveness of holdfast development in Oedogonium depended upon the roughness of the surface with which the zoospore came into contact before germinating. Fritsch (10) records that the rhizoidal ends of germinated zoospores of *Oedogonium capillare* develop much more slowly than the tip ends, the process requiring several hours.

My investigations with Spirogyra, Zygnema, and Oedogonium in continuous artificial illumination show that the rhizoids, when they occur, in these algæ are always negatively phototropic but not necessarily dependent upon a rough surface. Artificial illumination in some way causes rather excessive holdfast-like development in *Spirogyra majuscula*, *S. porticalis*, and *S. varians* from the ordinary vegetative cell. Bulges occur and develop from the middle of the cells which have every appearance of initial conjugation. The tubes develop very long and tortuous, sometimes branching, but never conjugate. The chloroplast pattern in these cells is much disarranged, sometimes is lost, and frequently no cross walls occur in the branch.

Pithophora varia in continuous artificial illumination exhibits some peculiarities of development that necessitate special mention. The first week of January, 1922, some healthy plants of this alga, found growing in the greenhouse under the diffuse sunlight of winter, were transferred to the constantly illuminated water. The initial stages of the formation of resting spores (sometimes incorrectly termed "akinetes") were observable in a few cells. Within a week after the transfer rapid movement of the main mass of cytoplasm and chloroplasts to the ends of the segments was noticed. The major movement was toward the swollen upper end of the segment. Starch and oil accumulation increased. A transverse wall formed separating the spore from the rest of the segment. If further movement of chloroplasts and cytoplasm continued after the completion of the first transverse wall, a second, a third, and even a fourth wall was laid down in rapid succession. Thus the spores occurred singly, in twos, in threes, or in fours. Very rarely a series of five resting spores are formed at the upper end of the segment. This process was completed within five days after the Pithophora was placed in artificial illumination. Mature spores in the tank from which the algæ were taken were not formed for two months afterward. The time required for the maturing of a spore is thus materially lessened by placing this alga in continuous artificial illumination.

It has been possible to follow the life cycle of this Pithophora still further under artificial light. After a dormant period of from four to six weeks the spores thus formed began to germinate. The old segments from which they were formed

were still alive, but showed no signs of rejuvenated activity. Under ordinary conditions of light the spores usually germinate in opposite directions from the two poles. A transverse wall early develops, the lower half giving rise to a more or less extensive holdfast, the upper half becoming the main part of the thallus. The spores under artificial illumination always germinate from opposite equatorial regions, at right angles to the direction from which the light comes. That is, there occurs a change in the polarity of the spore. One region germinates from two to five days sooner than the other. Neither region takes on the characteristics of a holdfast, and no transverse wall occurs in the spore. The two oppositely developing branches grow very long (sometimes three or four millimeters) before segmentation occurs.

Although initial germination is always at right angles to the direction from which the light strikes the spore, there is a subsequent turning of the upper end of the thallus toward the light. In a saturated atmosphere the upper ends sometimes extended above the surface of the water as much as a centimeter or two.

The segments produced from these spores began the formation of a second generation of spores in three weeks. The same movement of chloroplasts to the upper end of the segments, the formation of transverse walls, and the maturing of the spores were identical with the formation of the first spores. Under natural conditions spores are produced usually but once a year, and undergo a rather long period of dormancy. Under continuous artificial illumination the whole cycle from spore to spore was effected in three weeks.

The effects of artificial illumination upon so-called sexual conjugation have been observed in only one instance. *Spirogyra majuscula* in nature is a late spring or early summer annual, found in a fruiting condition usually from the last week in June to the middle of July. Some material of this alga, collected in early April of this year in a vegetative state, was placed in the artificially illuminated chamber at that time. Mature zygospores were observed on April 24, at least six weeks before the usual time out-of-doors. In the same tank, but not in the constantly illuminated box, the same alga four weeks after that date, showed no indications of conjugation.

From the two observations on *Pithophora varia* and *Spirogyra majuscula* noted above, one is led to conclude that the time

required to produce resting spores and zygotes in these two algæ was materially shortened. It must be stated, however, that the temperature of the water within the box is slightly higher than that outside. Just what effect this slightly higher temperature has, has not been ascertained.

The cell walls of Cladophora, Pithophora, Cylindrocapsa, and Tribonema are notably lamellated. It has been suggested that these lamellations may have some relation to the daily periodicity of sunlight. I have been unable to get any very conclusive data bearing one way or the other on this phenomenon. The lamellations in Cladophora are less in number and appear later under continuous artificial illumination, but are never absent. No appreciable differences were noted in the other algæ. The lamellæ are apparently a growth phenomenon not directly related to sunlight.

It was noted in a previous part of this paper that in Vaucheria the principal food reserve is oil. Species of *Vaucheria germinata* and *V. hamata* in continuous artificial illumination stored starch instead of oil. Sometimes there were both starch and oil present at the same time in the coenocyte. In those parts of the coenocyte developed after the plant was placed under artificial illumination starch was the predominant, sometimes entire, food reserve. These species of Vaucheria were grown both on moist soil and in water, and the results were practically identical as far as starch formation is concerned.

Some effects of calcium on colonial and filamentous integration. Some cultures of Spirogyra, Cladophora, Cylindrocapsa, Tribonema, and Chlorella in Knop's solution with or without calcium were kept in the greenhouse during the winter of 1922-23. Rayss (30) has observed that calcium salts in the proportion of .25 to 1.75% greatly favored coenobe production in *Coelastrum proboscideum;* lesser amounts tended toward coenobic disarticulation.

In cultures of Knop's solution without calcium the following observations were made. Checks with calcium in the solution showed practically normal growth.

Spirogyra.....Filaments either broke up into fragments or developed abnormally long cells. Cross wall formation inhibited. Middle lamella often partially formed.
Cladophora.....The plants grew practically normally.
Cylindrocapsa..The cells rounded and divided uniformly into four ellipsoid divisions, which never became zoospores. No cell division noted thereafter.
Tribonema.....Filaments extremely fragmentary, the isolated H-pieces being very common.
Chlorella.....The plant lost practically all semblance of a colonial aggregation.

I have noted elsewhere that microchemical tests for calcium in many of the green algæ gave negative results. The growth in these cultures contributes no proof as to the presence of calcium pectate in the algal cells, but in some cases it is shown that the absence of calcium in the surrounding media is responsible for colonial and filamentous disintegration. Apparently calcium is necessary for the formation of the middle lamella in these algæ. It remains to be determined whether this is a direct or an indirect effect on the formation of pectose.

Wall formation and permeability to copper sulphate.—It has been known for a number of years that most green algæ are rapidly killed by extremely dilute solutions of copper sulphate. Algæ quite resistant to the effects of this salt are Cladophora and Pithophora; less resistant are the Oedogoniales; and very susceptible are the Zygnemaceæ. Copper sulphate in the proportion of one part in a million parts of water is fatal to most Zygnemaceæ. It requires a concentration four times as great to kill Cladophora or Pithophora. Most Oedogoniales can withstand a concentration only twice as great.

A study of the nature of the cell walls of these algæ offers some explanation for their varying resistance or susceptibility to copper sulphate. The cell wall of the Zygnemaceæ is made up peripherally of a layer of pectose; most Oedogoniales have an outer layer of chitin; the cell walls of Cladophora and Pithophora are heavily chitinized on the outside. Young branches of the latter alga are much more readily killed than the older parts of the thallus. An examination of the cell walls of the younger parts shows, however, that chitinization has not been effected, or at least incompletely so.

It appears, then, that resistance to copper sulphate in the water is related to the amount of chitinization of the algal cell walls. Pectic compounds and cellulose are readily permeable to copper sulphate; chitin is much more difficultly permeable to the same copper salt.

Summary Statements.

1. Among species of Zygnemaceæ studied the cell wall of the filament in the vegetative state is uniformly of cellulose, surrounded by a layer of pectose of varying thickness.

2. The outer layer of the vegetative cell of Cladophora, Pithophora, and species of the Oedogoniales is chitin.

3. The zygospores and aplanospores of the Zygnemaceæ have three or more cell wall layers: the inner cellulose, usually thinner than the others; the outer of cellulose, occasionally with peripheral pectose; and the middle of cellulose with irregular deposits of chitin. Ornamentations of the middle layer are associated with these chitinous deposits.

4. In *Spirogyra illinoiensis* and *S. stictica* the outer spore wall is pectose and the gametangial walls are similar in construction to those of the zygotes.

5. The layers constituting the thickened gametangial wall in *Debarya decussata* are of cellulose, not of pectose.

6. The middle lamella of the cross walls of Spirogyra, Mougeotia, and Zygnema is uniformly of pectose, completely enveloped by cellulose.

7. Fragmentation of a filament in the Zygnemaceæ is brought about by the transformation of the pectose of the middle lamella into water-soluble pectin.

8. The cellulose H-pieces of *Microspora Willeana* are joined at their extremities by pectose or occasionally by calcium pectate. An inner layer of cellulose, independent of the H-pieces, surrounds the reticulated chloroplasts. The amount of pectose material present in the cell walls decreases with the age of the filament.

9. The lamellations of the cell walls of Cylindrocapsa are of cellulose. The whole is surrounded by a pectose sheath.

10. The cell walls of Tribonema have correspondingly lessened amounts of pectic compounds with increased age. The cell wall construction is very similar to that of *Microspora Willeana* described above.

11. Epiphytic algæ are usually attached to the host plant by a gelatinous layer of pectose.

12. In the Oedogoniales the outer chitinous layer is not present in those parts of the holdfasts and nannandria attached to the host, thus making the pectose layer peripheral at the points of attachment.

13. Epiphytic algæ do not usually occur on vegetative filaments of the Zygnemaceæ until there is a cessation of the transformation of pectose into water soluble pectin. This transformation usually ceases with initiation of reproductive activities.

14. Starches and oils are the principal food reserves of the green algæ studied.

15. Tests for sugars were not conclusive, although there is some evidence of the presence of glucose at the periods of greatest photosynthetic activity.

16. Color reactions for the pentosans, araban and galactan, were positive. No evidence of other hemicelluloses was secured.

17. Inulin tests were negative.

18. Tannins were not found in the green algæ during the vegetative period. Upon the initiation of reproduction the amount of tannin increases rapidly. The amount decreases during spore formation and there is scarcely a trace of tannin in the mature zygote.

19. Iron is present as ferric compounds in the chloroplasts.

20. Potassium is noted chiefly in the Zygnemaceæ at the time of conjugation in the region of the appressed areas of the conjugation tubes.

21. Calcium was observed in the larger Oedogoniales and occasionally in Tribonema. This mineral appears to bear some relation to the formation of the pectose middle lamella in the Zygnemaceæ, but tests for calcium in these algæ were negative.

22. Continuous artificial illumination reduces the life cycle period of *Pithophora varia* to three weeks; changes the polarity of the germinating resting spores ("akinetes"); and inhibits rhizoidal formation in this alga.

23. Constant artificial illumination causes the formation of mature zygotes in *Spirogyra majuscula* from six weeks to two months earlier than out-of-doors.

24. Lamellations in cell walls, whether of cellulose or of pectose, are not materially altered by constant artificial illumination.

25. The food reserve of species of Vaucheria under constant artificial illumination may be partially or wholly starch.

26. The absence of calcium from Knop's solution used as a culture medium produced colonial and filamentous disintegration in the algæ studied.

27. Calcium seems to have some necessary effect upon the initiation of cross walls in the septate filamentous algæ.

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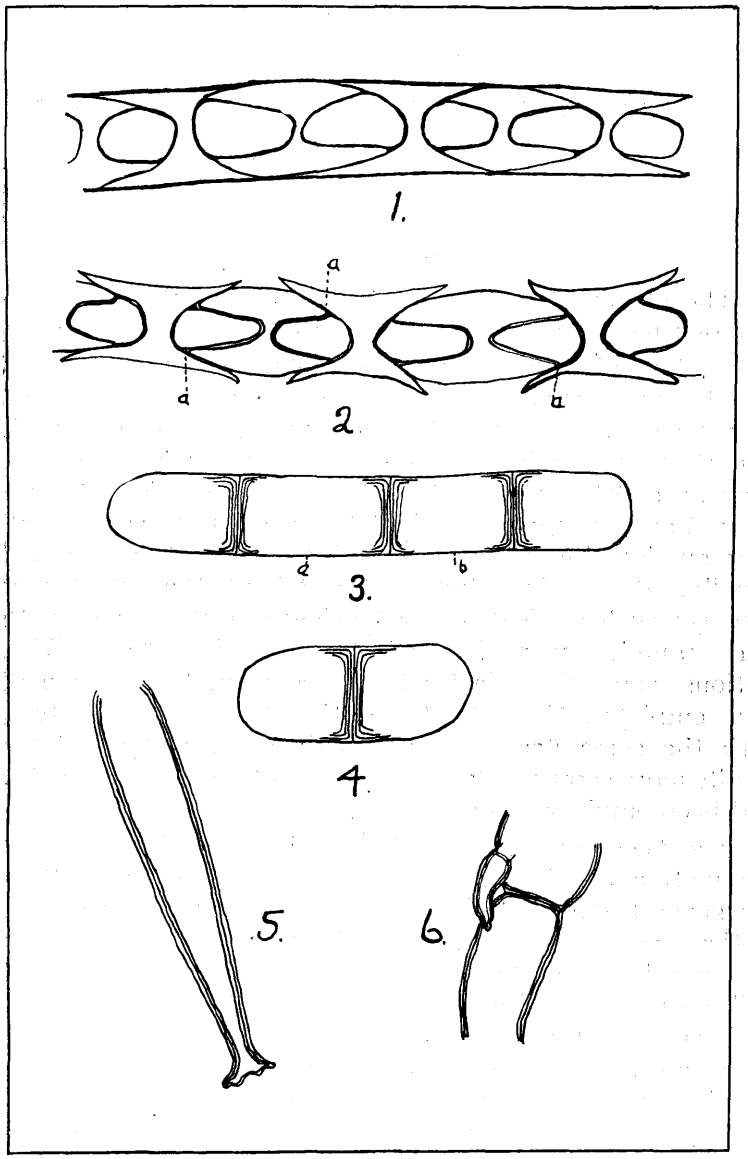

Fig. 1. Filament of *Microspora Willeana* Lagerh. under normal conditions.
Fig. 2. Same after treatment with warm HCl and KOH.
Fig. 3. Sporeling of *Microspora Willeana* Lagerh. with first transverse wall and pectose thickenings.
Fig. 4. Further transverse divisions of sporeling, indicating (a and b) where next cross walls will be laid down.
Fig. 5. Basal cell of *Oedogonium exocostatum* Tiffany showing dual nature of cell wall at points of attachment; triple nature elsewhere.
Fig. 6. Nannandrium on suffultory cell of *Oedogonium concatenatum* (Hass.) Wittr. showing dual nature of cell wall at points of attachment; triple nature elsewhere.

(All somewhat diagrammatic; see text for further explanation).